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ECO-FRIENDLY METHODS FOR TORULARHODIN EXTRACTION AND INCORPORATION INTO LIQUID SOAP FORMULATION

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ABSTRACT

Torularhodin, a potent member of the carotenoids xanthophylls class, is esteemed for its remarkable antioxidant, antimicrobial, and anticancer attributes. Despite its therapeutic promise, widespread production and commercialization remain challenging. This study investigates environmentally friendly methods for torularhodin extraction from *Rhodotorula glutinis* biomass derived from submerged cultivation. The efficiency of carotenoid extraction from wet biomass was explored for both mechanical and non-mechanical techniques. Notably, treatment involving acetone and ball mill demonstrated promising results, highlighting the importance of innovative extraction methodologies. Furthermore, the application of the carotenoid-rich extract in liquid soap formulation revealed alterations in physicochemical properties of soap, including pH and viscosity, alongside enhanced antioxidant activity. These findings underscore the potential of torularhodin-enriched products not only to enhance product characteristics but also to provide additional health benefits. This research signifies a step forward in the quest for sustainable extraction techniques and novel applications of bioactive compounds.

Keywords: Extraction techniques. Sustainable processes. Bioactive compounds. Downstream processing.

1 INTRODUCTION

Torularhodin, a carotenoid member of the xanthophylls class, which has remarkable antioxidant activity, as well as its antimicrobial and anticancer properties. Despite its therapeutic potential and promising applications across various fields, achieving large-scale production and commercialization of this compound remains elusive[1,2]. Therefore, research and development of techniques to obtain this carotenoid emerge as a crucial field of study to be explored.

Carotenoids are synthesized intracellularly by oleaginous yeast such as *Rhodotorula glutinis*. Following the cultivation phase, a series of unit operations in the downstream process becomes essential, including cell disruption, and purification steps, to obtain the molecule in its final form. Traditionally, downstream processing heavily relies on volatile organic solvents (VOCs), which are non-renewable and pose risks to human health and ecosystems. Recognizing this, the total or partial replacement of VOCs with more environmentally friendly and safer solvents is becoming imperative, aligning with the growing global demand for advanced technologies and sustainable processes[3].

Solvents derived from renewable biological sources mostly exhibit minimal and, for the most part, exhibit minimal toxicity and reduced environmental impact. However, to compete with VOCs, which often have attractive market prices, these solvents also need to possess a high solvency capacity and efficiency in extracting the target molecule[4].

The objective of this study was to explore methods for extracting torularhodin from *R. glutinis* biomass using mechanical and non-mechanical techniques, specifically chemistry through environmentally friendly solvents and physics, in addition, to use the carotenoids obtained from the microbial biomass as colorants in the formulation of liquid soap.

2 MATERIAL & METHODS

2.1. Production, extraction, and quantification of carotenoids

The yeast used was *R. glutinis* CCT-2186, acquired from the Tropical Culture Collection André Tosello (Campinas, SP, Brazil). Cultivation was carried out in a stirred tank bioreactor (Minifors II) equipped with two Rushton impellers submerged in the liquid, placed 14 cm apart, with a working volume of 4L, in three stages: pre-inoculum, inoculum, starter culture, and cultivation.

For the pre-inoculum, *R. glutinis* was grown on solid YPD-A medium. A loop of the microorganism was inoculated onto a YPD-A plate and incubated at 30 °C for 48 hours. The starter culture was prepared in an orbital shaker (Tecnal, model TE-421, Piracicaba, SP, Brazil) for 48 hours at 170 rpm and 30 °C, using an initial optical density (OD) of 0.1 at UA_{600nm} of fungi taken from the inoculum and transferred to 50 mL of starter culture medium in 500-mL Erlenmeyer flasks. Then, a volume of 400 mL of the starter culture was transferred to the bioreactor, completing a volume of 4L with culture media.

The starter culture medium (inoculation) consisted of (g/L): Dextrose (10), Bacteriological peptone (5), Yeast extract (3), and Malt extract (3). The culture media in the bioreactor consisted of Dextrose (10), KH_2PO_4 (0.52), $MgSO_4$.$7H_2O$ (0.52), N_4NO_3 and

Asparagine (10), with pH adjusted to 5.0. The bioreactor vessel containing the cultivation medium was sterilized at 121 °C for 30 minutes.

Cultivation in the bioreactor was continuously monitored using pH, dissolved oxygen, and temperature probes. Samples were taken every 24 hours to evaluate carotenoid production and glucose concentration over 144 hours.

Afterwards, the fermented medium was centrifuged at 10600 x*g* for 5 minutes at 4°C. The clarified broth was used to determine the final glucose concentration, while the pellets containing carotenoid-rich biomass were washed three times with a monobasic phosphate buffer (0.2 M) solution at pH 7, then frozen for 24 hours.

Five treatments were used to evaluate the extraction of carotenoids from microbial biomass: T1 - Thermal at 65°C + ethanol:ethyl acetate [67:33 v/v] with agitation for 1 h; T2 - Ultrasound with 5 pulses of 5 min. each and, after each pulse, 1 min. on ice + ethanol:ethyl acetate [67:33 v/v]; T3 - Thermal at 65°C + ethanol:ethyl acetate: H_2O [55:24:21 v/v]; T4 - Ultrasound with 5 pulses of 5 min. each and, after each pulse, 1 min. on ice + ethanol:ethyl acetate: H_2O [55:24:21 v/v]; T5 - Acetone 5 mL + ball mill. The experiments were conducted at a solid:liquid ratio of 0.2 g wet biomass/mL solvent.

Carotenoid quantification was carried out based on the absorbance spectrum using a UV-Vis spectrophotometer (model Genesis 10S, China) at a wavelength of 480 nm corresponding to torularhodin maximum absorbance and expressed as Units of Absorbance (UA).

2.2. Application of the carotenoid-rich extract in liquid soap formula

The transparent liquid soap base was purchased from a local shop in the city of Araraquara – SP, Brazil. A predetermined amount of carotenoid extract (0.2 g) was then added to 10 ml of liquid soap and stirred for 2 min. Continuous mixing was maintained to ensure efficient formulation. Subsequently, the liquid soaps were analyzed, resulting in a final concentration of 2.07 mg carotenoids per gram of soap. As a control, the liquid soap base was evaluated alongside the modified soap samples. The samples were assessed for density, pH, foam level, viscosity, and light stability.

2.3. Statistical analysis

The results were analyzed using Statistical Software 12.0 (StartSoft Inc., Tulsa, OK, USA). For assays of carotenogenic extract, the results underwent analysis of variance (ANOVA), and means were compared using Tukey's Test, both at a 95% confidence level.

3 RESULTS & DISCUSSION

3.1. Solid-Liquid extraction of carotenoids

Carotenoids sourced from yeast possess an inherently hydrophobic character, necessitating their extraction typically from dry biomass employing organic solvents like acetone, DMSO, and others[5]. In this study, akin to the methodology explored by Naveira-Pazos[6], we employed wet biomass instead of dry samples. This innovative approach holds considerable promise, potentially yielding substantial cost reductions by circumventing the requirement for biomass drying prior to analysis, thus streamlining operational procedures. The results are shown at Figure 1 which highlights treatment 5 as particularly noteworthy compared to the others evaluated. This could be attributed to the efficacy of cell disruption using glass beads, which effectively ruptures yeast cells, releasing intracellular compounds[7].

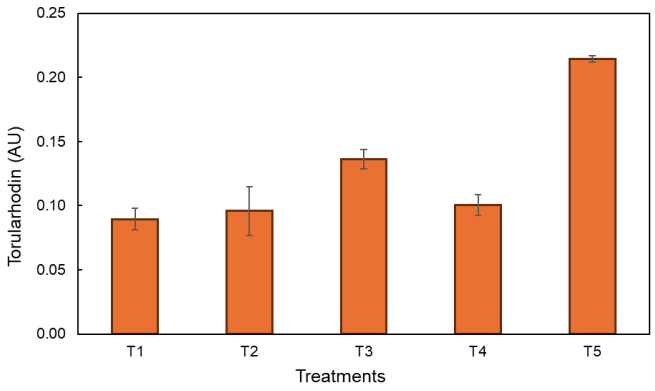

Figure 1 Extraction of Torularhodin by different treatments (T_1 - Thermal at 65°C + ethanol:ethyl acetate [67:33 v/v] with agitation for 1 h; T_2 - Ultrasound with 5 pulses of 5 min. each and, after each pulse, 1 min. on ice + ethanol:ethyl acetate [67:33 v/v]; T_3 - Thermal at 65°C + ethanol:ethyl acetate: H_2O [55:24:21 v/v]; T_4 - Ultrasound with 5 pulses of 5 min. each and, after each pulse, 1 min. on ice + ethanol:ethyl acetate: H_2O [55:24:21 v/v]; T_5 - Acetone 5 mL + ball mill). Means followed by the same letter do not differ statistically (α=0.05).

In a study conducted by Naveira-Pazos[6], researchers observed superior outcomes by combining EtOH, EtOAC, and H_2O (in a ratio of 67:33:00, w/w/w) with dry biomass. However, our study did not replicate this behavior, which could be attributed to the difference in biomass moisture content. The presence of H_2O, while necessary for solubilizing wet biomass, poses challenges for miscibility and solubilization with organic solvents. This presence also reduces cell wall permeability, which could account for the lower extraction efficiency observed in treatments 3 and 4. Additionally, the resistance of yeast cell walls suggests that physical (temperature) and mechanical (ultrasound) methods outperform the glass bead accretion method.

3.1. Application of the carotenoid-rich extract in liquid soap formula

Upon analyzing the formulation, we observed a uniformity in the carotenoid distribution, lending the product an appealing and consistent appearance. Furthermore, there was an increase in pH by 0.17 units compared to the control, suggesting a potential influence of carotenoids on the chemical composition of the liquid soap. Conversely, the formulation's density exhibited a decrease of 0.53 units, indicating a potential alteration in concentration or composition of its constituents.

In terms of apparent viscosity, the liquid soap infused with carotenoid extract displayed a value of 1790.80, whereas the control exhibited 2057.27. This variance in viscosity could be attributed to the interaction between carotenoids and other formulation ingredients, thereby impacting the final product's consistency.

Beyond the physicochemical attributes, the liquid soap underwent testing for DPPH free radical scavenging activity. Following the addition of both carotenoid-rich soaps and the control to the DPPH solution, a reduction in the initial absorbance spectrum was observed. Significantly, the carotenoid-enriched soap demonstrated heightened antioxidant activity, with a free radical scavenging rate of 22.91% compared to the control's 17.43%.

These findings underscore the efficacy of carotenoids such as torularhodin, and beta-carotene in acting as potent free radical scavengers. The confirmation of the intrinsic antioxidant properties of these carotenoids not only emphasizes their potential to enhance product characteristics but also to impart additional skin health benefits to users.

4 CONCLUSION

This study demonstrates the feasibility of extracting torularhodin in a sustainable manner using environmentally friendly solvents. The mechanical and non-mechanical techniques explored yielded promising results, underscoring the importance of innovation in the extraction of bioactive compounds. Additionally, the application of the carotenoid-rich extract in the formulation of liquid soap resulted in significant alterations in physicochemical properties, accompanied by enhanced antioxidant activity.

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